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Effect of Polyriboinosinic-Polyribocytidylic Acid and Antibody on Infection of Immunosuppressed Mice with Vaccinia Virus

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The effects were studied of polyriboinosinic-polyribocytidylic acid (poly I:C) and specific antiviral antibody on the course of fatal systemic vaccinia virus infection in immunosuppressed mice. Administration of a single dose of vaccinia-immune serum was more effective in protecting Cytoxan-treated mice on day 3 or day 4 after infection with vaccinia virus than were four daily doses of poly I:C begun on the same day. An antiviral effect of poly I:C, as determined by lower levels of viremia, was present within 24 hr after initiation of therapy with poly I:C. Combined therapy with poly I:C and antibody was considerably more effective than therapy with either of these agents alone when administered late in the course of this systemic infection. This suggests that combined therapy with an inducer of interferon and specific antibody to the virus might be effective in immunologically incompetent hosts with severe viral infections.

Increased severity of viral infections has been reported in immunosuppressed and immunologically deficient persons [1–5]. Such persons would be candidates for treatment with an interferon stimulator, such as polyriboinosinic-polyribocytidylic acid (poly I:C), and/or specific antiviral antibody. We have previously reported that therapy with poly I:C reduced the mortality of fatal systemic vaccinia virus infection in immunosuppressed mice [6]. In this study we describe the comparative and additive effects of poly I:C and specific antiviral antibody on the course of fatal systemic vaccinia virus infection in immunosuppressed mice.

Materials and Methods

Mice. NIH general purpose Swiss male mice (22–26 g) were used in all experiments.

Virus. Vaccinia virus was obtained from Dr. W. A Cassel, Emory University, Atlanta, Ga. Vaccinia virus was grown on the chick embryo chorioallantoic membrane; the pool titered 10^8 LD_{50}/ml when inoculated intracranially (ic) into weanling Swiss male mice and 10^9 pfu/ml when assayed on Vero cells.

Neutralizing antibody tests. Individual sera of mice were assayed for antibody to vaccinia virus by a plaque-reduction method. Samples of vaccinia virus containing 50 pfu/0.2 ml were incubated with 0.2 ml of threefold dilutions of serum in Eagle's medium no. 2 with 2% fetal bovine serum (FBS) at 37 C for 2 hr; each mixture was then plaque assayed on Vero cells. Vero cells were obtained from Dr. John Rhim of Microbiological Associates, Bethesda, Md. and were grown in Hanks' minimal essential medium with 10% FBS. A control sample of vaccinia virus incubated with threefold dilutions of normal murine serum was included in each experiment.

Female rabbits (2 kg) were given three sc injections of vaccinia virus (10^8 pfu of virus per injection) during a three-week period. A week after the final injection, they were bled by cardiac puncture, and the sera were collected and pooled.

Poly I:C. Double-stranded poly I:C was prepared as described previously [7]. Final concentration of this material was 0.5 mg/1.0 ml.

Cyclophosphamide. Cyclophosphamide (Cytoxan) was obtained from Meade Johnson, Co., and a solution in phosphate-buffered saline (PBS) with a final concentration of 20 mg/ml was prepared just before use.

Assay for vaccinia virus. Individual mice

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were bled by the orbital technique [8], and serial 10-fold dilutions of each serum in Eagle's medium no. 2 with 2% FBS were assayed for vaccinia virus by plaque titration on Vero cells.

Results

(1) Comparative effect of poly I:C and antiviral antibody on infection. In all studies of protection, mice were inoculated iv with 10^4 pfu of vaccinia virus in 0.1 ml of Eagle's medium no. 2 containing 2% FBS; 24 hr later they received 150 mg/kg of body weight of Cytoxan ip in 0.3 ml of PBS. In each experiment 20 mice that were infected with vaccinia virus and treated with Cytoxan were kept as controls, while groups of 20 mice were treated with four daily ip doses of 100 μg of poly I:C beginning three or four days after inoculation of virus. Similar groups of 20 mice were treated with a single dose of 0.4 ml of vaccinia-immune serum on day 3 or day 4 after inoculation of virus. As reported previously, administration of this dose of immune serum results in titers of antibody of 30–70 in sera of recipient mice, and these titers persist for at least three days [9]. The same pool of immune serum was used in the present study as was used in our previous studies [9]. Groups of 20 mice also received only vaccinia virus or only Cytoxan in each experiment.

When the data from the groups on days 3 and 4 is combined, it is evident that a single dose of immune sera was significantly more protective than four daily doses of poly I:C begun on the same day ($P < .01$) (figure 1). The poly I:C begun on day 3 or day 4 after infection did significantly delay death ($P < .01$ on days 10 and 12 after infection) but did not result in a significantly lower mortality at the end of the experiment.

(2) Effect of poly I:C. Poly I:C has been shown to be an immunologic adjuvant of both humoral and cellular immunity [10]; therefore, it seemed possible that poly I:C might have exerted its antiviral effect by some means other than the induction of interferon. In an attempt to test this possibility, studies were done to determine viremia and levels of serum antibody in immunosuppressed mice infected with vaccinia virus and treated with poly I:C. Mice that received their first dose of poly I:C on day 4 after infection had levels of viremia 24 hr after this first dose of poly I:C that were about 1/10 the levels of viremia in control mice not treated with poly I:C (figure 2). By the seventh day after infection, poly I:C-treated mice had levels of viremia that were 1/100 the levels of viremia in mice not receiving poly I:C. These results indicate that therapy with poly I:C rapidly suppressed the level of viremia in immunosuppressed mice.

Two immunosuppressed mice infected with vaccinia virus and treated with poly I:C were bled each day for determination of serum neutralizing antibody. No antibody could be detected in the

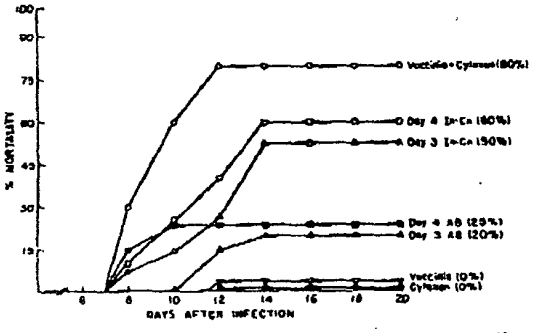

Figure 1. Effect of treatment with either polyriboinosinic-polyribocytidylic acid (In.Cn) or antibody (AB) on mortality from vaccinia virus infection in immunosuppressed mice.

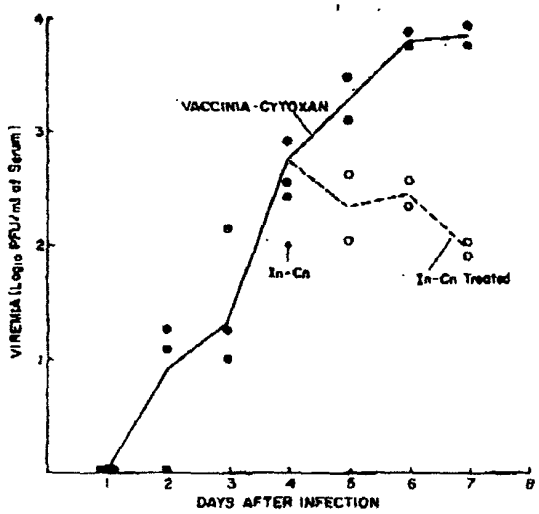

Figure 2. Effect of polyriboinosinic-polyribocytidylic acid (In.Cn) therapy on viremia in immunosuppressed mice infected with vaccinia virus.

serum of any of these mice until 14 days after infection, when one of two mice had about 50 units of antibody per ml. This strongly suggests that the fall in viremia within 24 hr after administration of poly I:C was not secondary to the stimulation of antibody formation by poly I:C.

(3) Effect of combined therapy with poly I:C and antiviral antibody. Since antibody and at least the interferon stimulated by poly I:C exert their antiviral effect by different mechanisms [11], it seemed of interest to determine whether their effects would be additive in the therapy of this experimental infection. The results of three experiments are summarized in table 1. In experiment no. 1, mice receiving both poly I:C and antibody had a lower mortality than mice receiving antibody only, but this difference is not statistically significant. Because antibody exerted such a significant antiviral effect when administered on day 5 after infection, in the subsequent two experiments antibody and poly I:C were administered on day 6 after infection. In both of these experiments, combined therapy with antibody and poly I:C exerted a significantly more protective effect than either poly I:C or antibody alone ($P < .01$ when the two experiments are combined). The results indicate that therapy with specific antibody and poly I:C is considerably more effective than therapy with either of these agents alone when administered late in the course of this systemic infection.

Discussion

In the present study, administration of a single dose of vaccinia-immune serum was more effective in protecting Cytoxan-treated mice on day 3 or day 4 after vaccinia virus infection than four daily doses of poly I:C begun on the same day. Poly I:C has been found to have many effects in experimental animals in addition to that of stimulating the formation of interferon. It has been shown to be an adjuvant of both humoral and cellular immunity [10] and to exert a biphasic effect on reticuloendothelial clearance [12]. The failure to detect serum antibody in mice protected from fatal systemic vaccinia virus infection until 14 days after infection suggests that poly I:C did not exert its major antiviral effect by stimulating the formation of specific neutralizing antibody. An antiviral effect of poly I:C, as determined by lower levels of viremia, was present within 24 hr after initiation of therapy. Since previous studies from this laboratory have demonstrated that cellular immunity is not critically important in recovery from this particular experimental virus infection [9, 13], it appears unlikely that poly I:C exerted its protective effect by enhancing cellular immunity. In a previous study it was demonstrated that the response of interferon to poly I:C in mice was not suppressed by very large doses of Cytoxan [6]; it appears likely that a major antiviral effect of poly I:C was secondary to its stim-

Table 1. Effect of polyriboinosinic-polyribocytidylic acid (poly I:C) plus antibody on mortality from vaccinia virus infection in immunosuppressed mice.

Experiment no.	Therapy: Only vaccinia virus	Therapy: Only Cytoxan	Therapy: Vaccinia virus-Cytoxan control	Day therapy instituted*: Poly I:C† Day 5	Poly I:C† Day 6	Antibody‡ Day 5	Antibody‡ Day 6	Poly I:C plus antibody§ Day 5	Poly I:C plus antibody§ Day 6
1	0‖	0	90	50#	ND**	25††	ND	10††	ND
1	0	10	95	ND	80	ND	80	ND	35††
3	5	0	80	ND	65	ND	65	ND	20††
Average	2	3	88	50	73	25	73	10	27

* Mice were injected iv on day 0 with 10^4 pfu of vaccinia virus in 0.1 ml of Eagle's medium containing 2% fetal bovine serum; 24 hr later they received 150 mg of Cytoxan per kg of body weight.

† 100 μg ip daily for four days, beginning on the day indicated.

‡ 0.4 ml of vaccinia-immune serum ip on day indicated.

§ Same dosage as described in † and ‡.

‖ Percentage mortality.

# $P < .05$.

** ND = not done.

†† $P < .01$.

ulation of large amounts of interferon, but it is possible that other as yet undefined effects of poly I:C may also have played an important role in the observed antiviral effect.

Immunosuppressed and immunologically deficient persons have been noted to be particularly susceptible to fatal viral infections [1–5]. Progressive systemic vaccinia virus infection has been a particularly severe problem in individuals with depression of both antibody-mediated and cellular immunity [1]. Cytoxan suppresses both humoral and cellular immunity in the mouse [14, 15], and the fatal systemic vaccinia virus infection that Cytoxan-treated mice develop may therefore be a model for similar infections in immunologically deficient patients. This would more likely apply to patients whose disease is dependent on a continuing viremia than to those with locally progressive vaccinia necrosum. The markedly superior effect of combined therapy with poly I:C and antibody in this study suggests that combined therapy with specific antiviral antibody and an inducer of interferon might be effective in immunologically incompetent patients with severe viral infections involving prolonged viremia, where antibody is usually most effective. As previously reported, most immunosuppressed mice develop pox lesions on their tails by the fifth or sixth day after vaccinia infection [6]; thus, combined therapy with antibody and poly I:C is highly effective when administered after the appearance of definite signs of disseminated vaccinia infection. It should be noted, however, that at the present time there is no stimulator of interferon that is nearly as effective in man as poly I:C is in mice and certain other experimental animals.

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